



## The Effect of Turmeric Extract (*Curcuma longa* L.) As a Potential Anthelmintic on Reducing Endoparasites in Naturally-Infected Sheep

### Pengaruh Ekstrak Kunyit (*Curcuma longa* L.) sebagai Antelmintik terhadap Penurunan Endoparasit pada Domba yang Terinfeksi secara Alami

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**ABSTRACT.** Turmeric extract (*Curcuma longa* L.) has the potential to be a natural anthelmintic and alternative to commercial deworming drugs for naturally-infected local sheep. A completely randomized design was used to compare the effect of 5 different oral treatments of turmeric extract tablets at 0 (TET-0), 200 mg (TET-200), 400 mg (TET-400), 800 mg (TET-800), and commercial Oxfendazole 225mg (Oxfen-225) on reducing endoparasites (fecal egg counts, FEC) of naturally-infected local yearling ewes at day-0 (before treatments), day-7, day-14, and day-21 (after treatments) using 4 replicates (n = 4). At day-0, all experimental ewes were naturally infected by FEC *Strongyles* nematode ranging from  $85.0 \pm 32.8$  to  $638 \pm 230$  eggs/g. Meanwhile, FEC *Fasciola* spp. and *Paramphistomum* spp. (Trematode), *Moniezia* spp. (Cestode), dan *Eimeria* spp. (coccidia) were found a little in a small number of ewes. Therefore, further analysis focused on FEC *Strongyles*. On day-7, only Oxfen-225 and TET-800 treatments reduced ( $P < 0.05$ ) FEC *Strongyles* by 100% and 64%, respectively. There was no difference ( $P > 0.05$ ) in the increased or reduced percentages of FEC *Strongyles* infections among group treatments during day-14 and day-21 although only Oxfen-225 treatment showed a constant decrease. It seems that orally administering turmeric extract tablet at 800 mg have the potential to reduce FEC *Strongyles* in sheep by 64% although its anthelmintic potential is still weaker than commercial oxfendazole 225 mg.

**Keywords:** Anthelmintic, local sheep, nematode, turmeric extract

**ABSTRAK.** Ekstrak kunyit (*Curcuma longa* L.) mempunyai potensi sebagai antelmintik alami pengganti obat cacing komersial pada domba lokal yang terinfeksi cacing. Rancangan acak lengkap digunakan untuk membandingkan pengaruh 5 perbedaan perlakuan ekstrak kunyit dalam bentuk tablet dengan dosis: 0 (TET-0), 200 mg (TET-200), 400 mg (TET-400), 800 mg (TET-800), dan Oxfendazole komersil 225mg (Oxfen-225) terhadap penurunan jumlah telur cacing pada feses (*Fecal egg counts*, FEC) domba betina muda yang terinfeksi cacing secara alami pada hari ke-0 (sebelum perlakuan), hari ke-7, hari ke-14, dan hari ke-21 setelah perlakuan menggunakan 4 ulangan (n = 4). Pada hari ke-0, semua domba eksperimen terinfeksi secara alami oleh nematoda *Strongyles* sebanyak  $85.0 \pm 32.8$  sampai  $638 \pm 230$  telur/gram feses. Sedangkan FEC *Fasciola* spp. dan *Paramphistomum* spp. (Trematoda), *Moniezia* spp. (Cestoda), dan *Eimeria* spp. (Koksidia) hanya ada di beberapa domba saja dalam jumlah yang sedikit. Sehingga Analisa selanjutnya difokuskan kepada FEC *Strongyles*. Pada hari ke-7, hanya perlakuan Oxfen-225 dan Cur-800 yang dapat mengurangi ( $P < 0.05$ ) persentase FEC *Strongyles* sebanyak 100% dan 64%, secara berurutan. Tidak ada perbedaan ( $P > 0.05$ ) pada peningkatan atau penurunan persentase FEC *Strongyles* pada semua perlakuan selama hari ke-14 dan hari ke-21 walaupun perlakuan Oxfen-225 memperlihatkan pengurangan persentase FEC *Strongyles* secara konstan. Ekstrak kunyit dalam bentuk tablet pada dosis 800 mg mempunyai potensi menurunkan infeksi FEC *Strongyles* sekitar 64% pada minggu pertama, tetapi kemampuan antiparasitiknya masih di bawah oxfendazole 225 mg.

**Kata kunci:** Antelmintik, domba lokal, ekstrak kunyit, nematoda

## INTRODUCTION

The lamb's market in Indonesia is highly prospective as the country reached the 4th largest population in the world after China, India, and USA with about 238 million peoples (Indonesia Statistics Center, 2010). As the world's largest Muslim country, it is common that lambs demand climbs significantly on annual celebration days of Eidul Fitr and Eidul Adha. Also, the obligation of Aqiqah for Muslim parents increases the demand

for slaughtering sheep. However, this high demand has not been followed by a significant increase in local sheep production. This is due to the majority of sheep production is still dependent upon small-scale traditional farmers in villages who are commonly identified with poor managements, low animal performances, but high in endoparasites infections (Partoutomo, 2004).

Dorny et al. (1996) reported that Indonesian local sheep were majorly infected by nematodes at over 7500 worms and about 81.5% of the nematodes were *Trichostrongylus* spp. This high endoparasite infection in sheep in Indonesia may be caused by not only a hot and humid tropical environment being suitable for parasites but also

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poor farming management. Nieuwhof and Bishop (2005) reported that gastrointestinal parasites contributed to a higher cost for preventive, treatments, and lost performance than footrot, chlamydial abortions, toxoplasmosis, and scabies diseases, respectively. However, Indonesian small-scale local farmers may have a limited budget to deal with endoparasite eradication using a routine commercial treatment so that they may need a more affordable anthelmintic treatment. Parasites resistance against commercially available chemical drugs is also a challenge for researchers to find out a natural and safer alternative product to combating endoparasites infection in ruminants but it is continuously available and affordable.

Turmeric (*Curcuma longa* L.) extract has the potential to be an anthelmintic for ruminants (Afrin et al., 2016; Nasai et al., 2016; Ullah et al., 2017) especially for local sheep as turmeric can be easily and widely grown in villages at house or farmyards. Turmeric extract consists of two typical major bioactive compounds namely curcuminoids and terpenoids (Ishii et al., 2011; Lee et al., 2014; Afrin et al., 2016; Nasai et al., 2016; Ullah et al., 2017). In vitro larvicidal study using *Haemonchus* spp. by Nasai et al. (2016) reported that ethanol extract of *Curcuma longa* L. showed a significant anthelmintic activity resulting in about 78% worm mortality within 24 h of exposure at a concentration of 200 mg/ml. Pandey et al. (2018) also found that *Curcuma longa* L. extract by either methanolic or aqueous at 1 – 10 mg/ml indicated to cause paralysis and death of the *Haemonchus* spp. within 12 h of exposure in vitro. Meanwhile, the current in vivo study aimed to test the effect of oral administering of turmeric extract tablets at different doses on reducing endoparasites infection in naturally-infected local yearling ewes.

## MATERIALS AND METHODS

### Animals and Treatments

Twenty non-pregnant yearling ewes (of Garut breed, Decree of Indonesian Agricultural Minister No. 2914/Kpts/OT.140/6/2011) were

selected to be used in this experiment and fitted into a completely randomized design with 5 different treatments and 4 replications ( $n = 4$ ). The treatments were (1) ewes without oral administering of turmeric extract tablet (TET-0) as a negative control, (2) ewes with the oral administering of 200 mg of turmeric extract tablet (TET-200), (3) ewes with the oral administering of 400 mg of turmeric extract tablet (TET-400), (4) ewes with the oral administering of 800 mg of turmeric extract tablet (TET-800), and (5) ewes with oral administering of commercial anthelmintic containing 225 mg of oxfendazole (Oxfen-225) as a positive control.

All experimental ewes were obtained from traditional small-scale farmers in Lembur Jambu Kampong, Sukakarya Village, Banyuresmi subdistrict, Garut regency, West Java province, Indonesia. All ewes were selected to be naturally infected by endoparasites according to initial fecal egg counts (FEC) analysis. During the experiment, each ewe was placed in a traditional woody pen without a floor and only covered by straws. Each ewe was fed a similar cut and carry fresh grass at *ad libitum* level. The housing and feeding systems represented common traditional small-scale sheep farming in villages. The nutrient contents of the field grass can be seen in Table 1. Dry matter, ash, crude protein, crude fiber, and crude oils analyses were done using the method of the Association of Official Analytical Collaboration (AOAC, 2005) while total digestible nutrients (TDN) was calculated using a similar TDN equation to Ramdani et al. (2020). Gross energy was measured using a bomb calorimeter.

The experiment was running for 4 weeks. The first week was setting and adaptation while the last three weeks are the treatment period. In the morning before oral administering treatments (Day 0), the feces of each ewe were freshly collected and put in an icebox. The feces sample was collected and sent to the laboratory for endoparasite analyses on the same day. The analysis was repeated at seven, fourteen, and twenty-one days after treatment.

Table 1. Nutrients composition (% Dry matter or otherwise stated) of a cut and carry field grass used in the experiment.

	Dry matter (% fresh)	Ash (%)	Crude protein (%)	Crude fiber (%)	Crude oils (%)	Nitrogen free extract (%)	Total digestible nutrients (%)	Gross energy (Kkal/kg)
Grass	21.8	9.33	9.10	28.8	4.72	48.1	60.6	2994

### Turmeric Extract Preparation, Granules, and Tablets

Turmeric was watery cleaned and dried for a night. After that, turmeric was sliced into small pieces, blended with water in an electric blender, and squeezed. About 29 kg turmeric was extracted by using 10 L of water. Turmeric extract liquid was freeze-dried with the help of ethanol at  $-80^{\circ}\text{C}$  along the night. It was then to make granulated and tableted turmeric extract.

The dried extract was granulated by wet granulation method using ingredients as follow: dried extract (650 g), avicel (260 g), aerosol (30.3 g), LHPC (130 g), and alcohol sufficiently. Dried extract, avicel, and LHPC were mixed using geometrical technique until homogenized and aerosol was added and mixed again. After that, alcohol was sprayed little by little while mixing until the mixture is slightly wet. The mixture was then granulated using a sieve no. 12 and oven-dried at  $60^{\circ}\text{C}$  until its water content below 2%. Meanwhile, tablets were made by adding granules to several excipients such as talk (glidan), Mg-stearat (lubricant), and Ac-disol (crusher) using the formula: granules (93%), talk (2%), Mg-stearat (2%), and Ac-disol (3%). Granule mixtures were tableted to contain 200 mg of turmeric extract each.

### Fecal Egg Counts of Endoparasites

The FEC analysis to identify and count endoparasites was done in Animal and Veterinary Public Health Center owned by Food Security and Livestock Services of West Java government located in Cikole – Lembang. The center has been accredited by National Accreditation Committee of Indonesia (LP-331-IDN). McMaster egg counting technique was used in this FEC analysis as explained in Roepstorff and Nansen (1998) and Vadlejš et al. (2011). Briefly, about 4 g feces sample was transferred into a container and 56 ml of floating fluid (400 g NaCl and 500 g glucose in 1-liter distilled water) was added and stirred thoroughly using a spatula. The fecal suspension was then filtered through a tea strainer into a new container. Filtered suspension in a new container was stirred using a Pasteur pipette and using the same pipette withdrawn a sub-sample as the filtrate was being stirred. The first sub-sample was used to fill the first compartment of the McMaster counting chamber. The fluid was stirred again and pipetted to fill another sub-sample for the second chamber. It was then to leave the chamber to stand

for about 5 minutes to allow the eggs to float to the surface and the debris to go to the bottom of the chamber. After that, examined each sub-sample of the filtrate under a microscope at 10 x 10 magnification to identify and count all eggs within the engraved area of both chambers.

### Statistical Analysis

MINITAB 16 software was utilized in all statistical analyzed. The mean and its standard error (mean  $\pm$  SEM) of total eggs (eggs/g) of each endoparasite species in each treatment ( $n = 4$ ) was descriptively analyzed. Only FEC *Strongyles* spp. was further statistically analyzed because FEC *Fasciola* spp. and *Paramphistomum* spp. (Trematode), *Moniezia* spp. (Cestode), dan *Eimeria* spp. (coccidia) were seen a little in a small number of ewes. In this study, one-way analysis of variance (ANOVA) could not be used as the data did not pass the Anderson–Darling normality test at  $P > 0.05$ . Alternatively, the data were analyzed using Kruskal-Wallis and Mann-Whitney nonparametric tests to statistically compare the effect of different doses of turmeric extract treatments in sheep on FEC *Strongyles* spp. reduction (%) from 0 to 7, 14, and 21 days at  $P < 0.05$ .

## RESULTS AND DISCUSSION

Table 2 describes the means ( $\pm$  SEM) of FEC analyses (eggs/g) of several parasite infection in local yearling ewes treated by different doses of turmeric extract. As can be seen in Table 2, the levels and types of parasites infestation among local ewes varied considerably as explained by their high values of SEM. At day-0, all experimental ewes were naturally infested by FEC *Strongyles* nematode ranging from  $85.0 \pm 32.8$  to  $638 \pm 230$  eggs/g. Meanwhile, FEC *Fasciola* spp. and *Paramphistomum* spp. (Trematode), *Moniezia* spp. (Cestode), dan *Eimeria* spp. (coccidia) were seen a little in a small number of ewes. Therefore, further analysis focused on a reduction percentage of FEC *Strongyles* (Figure 1.) in the ewes after being treated by turmeric extract treatments.

Table 3 shows the means ( $\pm$  SEM) of FEC *Strongyles* infection (eggs/g) in local yearling ewes before and after turmeric extract treatments ( $n = 4$ ) along with increased or reduced percentages of infestation (%). On day-7, only Oxfen-225 and TET-800 treatments reduced ( $P < 0.05$ ) FEC *Strongyles* by 100% and 64%,

respectively. There was no difference ( $P>0.05$ ) in the increased or reduced percentages of FEC *Strongyles* infections among group treatments during day-14 and day-21 although only Oxfen-225 treatment showed a constant decrease.

Tabel 2. Means ( $\pm$  SEM,  $n = 4$ ) of FEC (eggs/g) of several parasite infections in local yearling ewes treated by different doses of turmeric extract tablets.

Treatments	Nematode <i>Strongyles</i> spp.	Trematode <i>Fasciola</i> spp.	Trematode <i>Paramphistomum</i> spp.	Cestode <i>Moniezia</i> spp.	Coccidia <i>Eimeria</i> spp.
Day-0	Eggs/g	Eggs/g	Eggs/g	Eggs/g	Eggs/g
TET-0	85.0 $\pm$ 32.8	2.50 $\pm$ 2.50	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
TET-200	488 $\pm$ 247	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	15.0 $\pm$ 15.0	0.00 $\pm$ 0.00
TET-400	638 $\pm$ 230	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	7.50 $\pm$ 7.50	0.00 $\pm$ 0.00
TET-800	415 $\pm$ 231	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
Oxfen-225	210 $\pm$ 114	2.50 $\pm$ 2.50	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
Day-7					
TET-0	100 $\pm$ 57.9	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
TET-200	728 $\pm$ 371	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
TET-400	645 $\pm$ 336	7.50 $\pm$ 7.50	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
TET-800	90.0 $\pm$ 34.6	5.00 $\pm$ 5.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
Oxfen-225	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
Day-14					
TET-0	75.0 $\pm$ 8.66	12.5 $\pm$ 12.5	0.00 $\pm$ 0.00	7.50 $\pm$ 7.50	0.00 $\pm$ 0.00
TET-200	360 $\pm$ 262	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
TET-400	563 $\pm$ 403	10.0 $\pm$ 10.0	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
TET-800	158 $\pm$ 64.1	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
Oxfen-225	45.0 $\pm$ 28.7	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
Day-21					
TET-0	330 $\pm$ 167	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
TET-200	330 $\pm$ 169	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	15.00 $\pm$ 8.66	0.00 $\pm$ 0.00
TET-400	2258 $\pm$ 987	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	7.50 $\pm$ 7.50	0.00 $\pm$ 0.00
TET-800	473 $\pm$ 304	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	7.50 $\pm$ 7.50	0.00 $\pm$ 0.00
Oxfen-225	45.0 $\pm$ 45.0	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00

SEM: standard error of the mean; n: number of replicates; FEC: fecal egg counts; TET-0: ewes without any anthelmintic treatment; TET-200: ewes with 200 g turmeric extract tablet oral treatment; TET-400: ewes with 400 g turmeric extract tablet oral treatment; TET-800: ewes with 800 g turmeric extract tablet oral treatment; Oxfen-225: ewes with 225 mg commercial oxfendazole oral treatment.

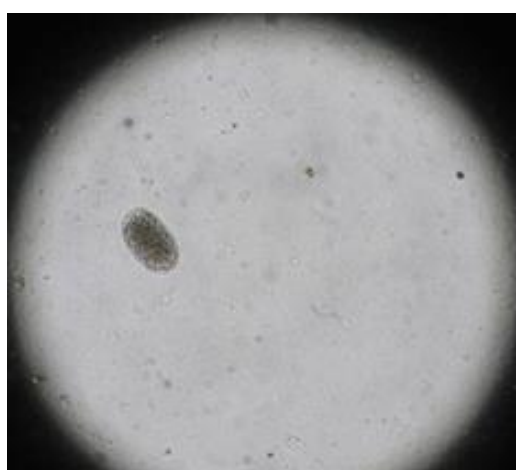


Figure 1. Egg of *Strongyles*

Table 3. Means ( $\pm$  SEM) of FEC *Strongyles* nematode infection in local yearling ewes before and after turmeric extract treatments (n = 4) along with increased or reduced infestation percentages (%).

Treatments	<i>Strongyles</i> spp. nematode (eggs/g feces)				Reduced (-) or increased (+) percentages (%)		
	Day 0	Day 7	Day 14	Day 21	Day 7	Day 14	Day 21
TET-0	30	60	90	0	100	200	-100
TET-0	70	10	60	570	-86	-14	714
TET-0	180	60	90	90	-67	-50	-50
TET-0	60	270	60	660	350	0	1000
Mean $\pm$ SEM					74.0 <sup>ab</sup> $\pm$ 101	34.0 $\pm$ 56.3	391 $\pm$ 275
TET-200	90	60	90	300	-33	0	233
TET-200	900	1470	1140	810	63	27	-10
TET-200	930	1260	30	30	35	-97	-97
TET-200	30	120	180	180	300	500	500
Mean $\pm$ SEM					91.3 <sup>a</sup> $\pm$ 72.4	108 $\pm$ 134	157 $\pm$ 134
TET-400	180	210	0	2040	17	-100	1033
TET-400	750	0	0	150	-100	-100	-80
TET-400	1230	900	540	1920	-27	-56	56
TET-400	390	1470	1710	4920	277	338	1162
Mean $\pm$ SEM					41.8 <sup>ab</sup> $\pm$ 82.0	21 $\pm$ 106	543 $\pm$ 323
TET-800	1030	150	60	90	-85	-94	-91
TET-800	510	150	330	1380	-71	-35	171
TET-800	60	30	60	210	-50	0	250
TET-800	60	30	180	210	-50	200	250
Mean $\pm$ SEM					-64.0 <sup>b</sup> $\pm$ 8.57	17.8 $\pm$ 63.8	145 $\pm$ 80.8
Oxfen-225	180	0	0	0	-100	-100	-100
Oxfen-225	540	0	60	0	-100	-89	-100
Oxfen-225	90	0	120	180	-100	33	100
Oxfen-225	30	0	0	0	-100	-100	-100
Mean $\pm$ SEM					-100 <sup>c</sup> $\pm$ 0.00	-64.0 $\pm$ 32.4	-50.0 $\pm$ 50.0
p value					P = 0.030	P = 451	P = 0.226

Means in the same column with different superscript differ significantly ( $P < 0.05$ ); SEM: standard error of mean; FEC, fecal egg counts; n: number of replicates; TET-0: ewes without any anthelmintic treatment; TET-200: ewes with 200 g turmeric extract tablet oral treatment; TET-400: ewes with 400 g turmeric extract oral treatment; TET-800: ewes with 800 g turmeric extract oral treatment; Oxfen-225: ewes with 225 mg commercial oxfendazole oral treatment

Routine use of chemical drugs as an anthelmintic to eradicate parasites at improper doses of administration in livestock will lead to parasite resistance. Treatment using natural anthelmintics derived from plants is an alternative to avoid resistance to chemical anthelmintics. The use of turmeric extract is an alternative to parasitic eradication that does not cause resistance because turmeric is a natural material that is non-toxic and friendly to the environment (Cervantes-Valencia et al., 2016).

It has been reported that turmeric extract is an anthelmintic that has the potential to combat endoparasites. Previous research by Nasai et al. (2016) showed that worms were affected by *Curcuma longa* L. extract in certain doses for a certain period time would experience paralysis and death because of acicolinoline nicotinic receptor depolarization in muscle nerves. *Curcuma longa* also provides antioxidant effects that interact with different signals of enzymes

involved in oxidative stress (Cervantes-Valencia et al., 2016). Ullah et al. (2017) reported that Thymoquinone and curcumin can suppress antioxidant activities such as *glutathione S. transferase* in adult flukes of *Fasciola gigantica*. Parasitic infections cause an increase in reactive oxygen intermediate production resulting in an imbalance condition between oxidants and antioxidants both *in vitro* and *in vivo* (Ullah et al., 2017). Also, parasites have well-developed antioxidants that allow them to survive in hostile environments produced by their hosts by neutralizing reactive oxygen species (Ullah et al., 2017).

Decreasing the number of FEC *Strongyles* due to TET-800 oral treatments could be caused by the effect of curcumin compound in turmeric extract on inhibiting the movement and suppressing the infective activity of the worms (Ullah et al., 2017). In addition to reducing the number of worm eggs, curcumin was also proven

to reduce the number of *Eimeria* sp. oocyst (Cervantes-Valencia et al., 2016). Also, Nasai et al. (2016) reported that *Curcuma longa* extract at 200 mg/ml gave the same effect as 1.5 mg/ml levamisole indicating that the extract has the potential to substitute levamisole as an anthelmintic against *Haemonchus* spp. larvae

## CONCLUSIONS

It can be concluded that oral administration of 800 mg of turmeric extract has the potential to combat FEC Strongyles spp. in sheep. The anthelmintic potency of 800 mg turmeric extract tablets is 64% lower than the 225 mg commercial oxfendazole.

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